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(57) Abstract

A method of prognosing in a head-injured subject or a subject who may be at risk of sustaining a head injury for the likelihood that a head injury might give rise to a chronic neurodegenerative pathology which could result in neuropsychological, psychiatric or neurological deficits, the method comprising detecting the presence or absence of ApoE isoforms or of DNA encoding ApoE isoforms in the subject.

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NOVEL METHOD OF PROGNOSING CHRONIC NEURODEGENERATIVE PATHOLOGY FOLLOWING A HEAD INJURY

The present invention relates to methods of prognosing the likelihood of neurodegenerative pathology and dementia in head-injured patients.

Accidental or non-accidental head injuries are common events. The precise number of patients suffering a head injury are difficult to calculate exactly since the methods of defining and counting cases varies from country to country. However the relevant figures for the UK serve as a useful guide to the extent of the problem. In the UK some 300 persons per 100,000 of the population are admitted to hospital each year as a result of head injury. Of these patients 9 per 100,000 will die as a direct result of the severity of their injuries. Outcome surveys in the USA indicate that for every 100 head injury survivors upto 5 remain in a coma, up to 15 are still severly disabled six months after injury, 20 have minor psychiatric or psychological problems and the remaining 60 will make a good recovery. These figures give rise to an estimated population of some 500, 000 persons in the USA who have a persisting handicap as a result of trauma related head injury. The social and economic cost of dealing with the after effects of such injuries is large[1,2,3,4].

The cause of this problem is the brain damage that occurs in up to 30% of patients who are admitted to hospital with a head injury[4]. The damage arises from the physical effects of the trauma (such as swelling, herniation, haemorrhage, global or focal damage or compromise of the vascular supply, contusion, cranial and peripheral nerve damage, axonal injury and embolism[3,4,5,6]) and also from the neurochemical consequences of the ischaemia which invariably accompanies physical brain damage[3,4,5,6]. Such injuries and subsequent damage are often widespread and can involve regions of the spinal cord, cranial and peripheral nerves in addition to the brain[1,3,4].

In addition the brain damage caused by head injury also produces the risk of subsequent psychiatric and neurologic complications including epilepsy and chronic neuro-degenerative states (eg dementia pugilistica or punch drunk syndrome)[3,4,5,6,7,8,9,10,11].

The extent of brain damage caused by a head injury can vary markedly from patient to patient. Thus, the likelihood and degree of sustaining pathological brain damage in the immediate aftermath of the trauma and the risk of subsequent chronic neurodegeneration leading to epilepsy or a dementing condition vary also.

The pathophysiology of head injury has been investigated in an effort to determine the molecular mechanisms which enable the acute triggering event of a head injury to be transformed into a chronic neurodegenerative pathological process[12,13,14,15].

Head-injured patients show increased levels of β amyloid precursor protein immunoreactivity[14] and some 30% of head injured patients have evidence of β amyloid protein deposition[15]. This deposition can occur within days of a single head injury. The eventual consequence of substantial numbers of β amyloid deposits is the emergence of a clinical syndrome of cognitive decline and increasing dementia[3,8]. Such deposits have been shown to be present in a number of dementing syndromes and these include Alzheimer's disease, cortical Lewy body disease, Parkinson's disease and the Alzheimer-type disease in patients with Down's syndrome[3]. In addition β amyloid deposits are present in the brains of patients with vascular and cerebrovascular disease and these latter conditions can predispose or contribute to the above diseases[3].

Recently, the apolipoprotein E (ApoE) genotype has been shown to be an important determinant in the etiology of AD[16-23] with the presence and number of E4 alleles being associated with increased risk and earlier ages of onset of disease in both familial cases linked to chromosome 19 and sporadic cases. The presence of E2 alleles has been claimed to decrease the risk (be 'protective') of late onset Alzheimer disease[18,19,24]. This inference is based on the increased frequency of ApoE4 alleles in patients known to have Alzheimer's disease and the later age at onset of disease in patients with the ApoE2/3 genotype compared to the ApoE4/4 genotype[24,25].

Such general 'protective' effects of the E2 allele have been reported previously in the general population with respect to heart disease[26,27].

The exact role of ApoE in the pathology of Alzheimer-type disease is uncertain. ApoE is co-localised with β amyloid within plaques in the central nervous system (CNS)[34] and has been shown to bind to β amyloid in vitro[35,36,37] and to tau proteins[28]. This has led to the hypothesis that ApoE/tau interactions are critical in the pathophysiology of tangle formation and thus central to the process of Alzheimer-type diseases[28]. However, neither parkinson dementia complex of Guam nor aged Down's syndrome patients show increased levels of ApoE4 alleles despite the presence of large numbers of tangles in the CNS[29,30]. As such the role of ApoE in the pathology of Alzheimer type dementia remains obscure.

The exact relationship of various environmental factors like head injury to subsequent degenerative conditions like Alzheimer's disease is uncertain. Although epidemiological studies provide some evidence for a link[9] the reason for the susceptibility to a chronic degenerative condition following head injury in some patients[8,9,10,11,15] is unknown at present.

Methods of diagnosing or prognosing Alzheimer's disease have been described (WO 94/09155) based upon detecting (directly or indirectly) the presence or absence of an apolipoprotein E type 4 (ApoE4) isoform in the subject. The ApoE alleles E2, E3 and E4 are described in the literature[31,32].

It has now been found that the frequency of ApoE4 alleles in those individuals with β amyloid deposition following head injury is of the same high order as that seen in Alzheimer's disease, while in those head injured individuals without β amyloid deposition, the ApoE4 allele frequency is similar to that in non-Alzheimer's disease controls.

This evidence provides the first explanation for the susceptibility of some patients to a chronic neurodegenerative pathological process following the types of brain damage (eg axonal shearing, swelling, herniation, hemorrhage and ischaemia) caused by a head injury.

The present invention therefore provides a method of prognosing in a head-injured subject or a subject who may be at risk of sustaining a head injury for the likelihood that a head injury might give rise to a chronic neurodegenerative pathology which could result in neuropsychological, psychiatric or neurological deficits, the method comprising detecting the presence or absence of ApoE isoforms or of DNA encoding ApoE isoforms in the subject.

The step of detecting the presence or absence of ApoE isoforms or of DNA encoding such isoforms may be carried out either directly or indirectly by any suitable means, such as by techniques well known in the art, and is preferably carried out *ex vivo* (eg by means of the method described[33,38]). All generally involve the step of collecting a sample of biological material containing either DNA or ApoE from the subject, and then detecting which isoforms the subject possesses from that sample. For example, the detecting step may be carried out by collecting an ApoE sample from the subject (for example, from cerebrospinal fluid, or any other fluid or tissue containing ApoE), and then determining the presence or absence of an ApoE isoform in the ApoE sample (eg, by isoelectric focusing or immunoassay). In the alternative, the detecting step may be carried out by collecting a biological sample containing DNA from the subject, and then determining the presence or absence of DNA encoding an ApoE isoform in the biological sample. Any biological sample which contains the DNA of that subject may be employed, including tissue samples and blood samples, with blood cells being a particularly convenient source. Determining the presence or absence of DNA encoding an ApoE isoform may be carried out with an oligonucleotide probe labelled with a suitable detectable group, or by means of an amplification reaction such as a polymerase chain reaction or ligase chain reaction (the product of which amplification reaction may then be detected with a labelled oligonucleotide probe). Further, the detecting step may include the step of detecting whether the subject is heterozygous or homozygous for the gene encoding an ApoE isoform. Numerous different oligonucleotide probe assay formats are known which may be employed to carry out the present invention. Suitable examples

of techniques and strategies for detecting the ApoE isoforms and encoding DNA are described in WO 94/09155.

It will be readily appreciated that the detecting steps may be carried out directly or indirectly. Thus, for example, any of the techniques described above for detecting ApoE2 may instead be used to detect ApoE3 and ApoE4. If either ApoE4 or ApoE3 is also detected in the subject, then it is determined that the subject is not homozygous for ApoE2; and if both ApoE4 and ApoE3 are detected in the subject, then it is determined that the subject is neither homozygous nor heterozygous for ApoE2.

The present invention has utility in enabling improvements in the clinical prognosis of patients who have suffered a degree of brain damage following a head injury.

In addition the invention has utility in allowing definition of the degree of risk in individuals who may be at risk of sustaining a head injury through social or professional activities (eg amateur and professional boxers, divers and other sportsmen such as rugby players, mountain climbers, judo players etc) or through elective medical procedures known to be associated with increased risk of brain damage (eg cardiac bypass operations, carotid endardectomy, brain surgery etc).

The method of the invention may thus be used to determine the degree of risk in participating in sporting events or clinical procedures. Such prognostications will have considerable utility in the design, planning and implementation of clinical care for patients in the event of a head injury and in the appropriate therapeutic intervention or the degree of hospital/social intervention or support required by a patient deemed to be at greater risk of a neurodegenerative disorder or in the design and analysis of clinical trials to determine the efficacy of therapeutic agents in the treatment of the types of brain damage which occur following head injury.

EXAMPLE

Method

Individuals surviving for less than two weeks following a severe head injury were selected from the Glasgow head injury database[4]. Most of the injuries were due to road traffic accidents or falls. Immunostaining for β-amyloid protein (β-AP) and Apolipoprotein E (ApoE) genotyping[38] were performed by standard methods.

Data

Deposits of β-amyloid protein resembling diffuse plaques were present in the cerebral cortex in 23 out of 90 (26%) individuals (Table 1). The ApoE-E4 allele frequency in those individuals with deposition of β-AP was 0.52 (Table 2a)

compared with 0.16 for those individuals without β-AP deposition (chi square 23.013: 1df, p<0.00001). This is similar to the previously published ApoE-E4 frequencies in individuals with Alzheimer's disease and age-matched control, respectively. Age stratification of the data indicates that the relationship between ApoE-E4 and β-AP deposition holds for those head-injured patients under 60 years of age (Table 2b). The proportion of head-injured individuals with β-AP deposition for each ApoE genotype is shown in Table 3. The proportion of head-injured individuals with β-AP deposition increased with the number of ApoE-E4 alleles (Table 4) from 10% for those without an E4 allele, to 35% for those with one E4 allele, to 100% (6 out of 6) for the relatively rare E4 homozygote. Within the group of patients with β-AP deposits, when the plaque density was assessed semi-quantitatively (sparse, moderate, frequent) it was found to be related to ApoE-E4 gene dose (Table 5).

Table 1 Descriptive statistics for head-injured individuals with (β-AP+) and without (β-AP-) β-AP deposition

		β-AP+	β-AP-
Number		23	67
Age (years)			
	mean ± SD	52 ± 19	28± 18
	range	14-75	0.15-79
Survival following head injury (days)			
	mean ± SD	3.3 ± 4	2.9 ± 3
	range	<1-13	<1-13

Table 2 ApoE allele frequencies in head-injured individuals with β-AP deposition (β-AP+) and without (β-AP-)

a. All head-injured patients

ApoE allele	β-AP+	β-AP-
E2	1/46 (0.02)	14/134 (0.1)
E3	21/46 (0.46)	98/134 (0.73)
*E4	24/46 (0.52)	22/134 (0.16)

*χ^2=23.013, 1df, p<0.00001

b. Head-injured patients under 60 years of age

ApoE allele	β-AP+	β-AP-
**E4	14/28 (0.5)	22/126 (0.16)

**χ^2=13.542, 1df, p<0.001

Table 3 Proportion of individuals with deposition of β-AP (β-AP+) according to ApoE genotype

ApoE genotype	Proportion of individuals β-AP+	Percentage
2/2	0/2	0%
2/3	0/7	0%
3/3	5/41	12%
2/4	1/4	25%
3/4	11/30	37%
4/4	6/6	100%

Table 4 Proportion of individuals with deposition of β-AP (β-AP+) according to the ApoE-E4 gene dose.

ApoE-E4 gene dose	Proportion of β-AP+ individuals	Percentage
0	5/50	10%
1	12/34	35%
2	6/6	100%

χ^2 for trend =22.85, 1df, $p<0.001$

Table 5 β-AP plaque numbers according to the ApoE-E4 gene dose.

ApoE-E4 gene dose	Proportion of head-injured patients with 'frequent' β-AP plaques	Percentage
0	0/5	0%
1	4/1212	33%
2	4/6	66%

χ^2 for trend $p=0.02$

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CLAIMS

1. A method of prognosing in a head-injured subject or a subject who may be at risk of sustaining a head injury for the likelihood that a head injury might give rise to a chronic neurodegenerative pathology which could result in neuropsychological, psychiatric or neurological deficits, the method comprising detecting the presence or absence of ApoE isoforms or of DNA encoding ApoE isoforms in the subject.

2. A method according to claim 1 wherein the step of detecting the presence or absence of ApoE isoforms or of DNA encoding such isoforms is carried out *ex vivo*.

3. A method according to claim 2 wherein said detection step involves collecting a sample of biological material containing DNA from the subject.

4. A method according to claim 3, wherein the biological sample is blood.

5. A method according to claim 2 wherein said detection step involves collecting a sample of biological material containing ApoE from the subject.

6. A method according to claim 5, wherein the biological sample is cerebrospinal fluid.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/02827

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68 G01N33/92

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DISSERTATION ABSTRACTS INTERNATIONAL B, vol. 54, no. 4, 1 October 1993 WASHINGTON DC USA, page 1827 D.M. VANDERPUTTEN 'Identification and characterization of apolipoprotein E in human neurodegeneration.' see the whole document ---	1-6
A	WO,A,94 09155 (DUKE UNIVERSITY) 28 April 1994 cited in the application see the whole document ---	1-6
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF NEUROLOGY, NEUROSURGERY AND PSYCHIATRY , vol. 57, 1 January 1994 LONDON UK, pages 419-425, G.W. ROBERTS ET AL. 'beta-Amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease.' see the whole document	1-6
T	NATURE MEDICINE, vol. 1, no. 2, 1995 WASHINGTON DC USA, pages 135-137, J.A.R. NICOLL ET AL ET AL 'Apolipoprotein E epsilon-4 allele is associated with deposition of amyloid beta-protein following head injury.' see page 135, column 1, line 7 - line 10	1-6

INTERNATIONAL SEARCH REPORT

...rmation on patent family members

International Application No
PCT/EP 95/02827

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		CN-A-	1092525	21-09-94
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